

(19)



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(11)

EP 0 925 723 A1

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
30.06.1999 Bulletin 1999/26

(51) Int. Cl.⁶: **A23J 3/14**, A23J 1/14,
A23K 1/16, A23K 1/18,
A23L 1/305

(21) Application number: 97310497.9

(22) Date of filing: 23.12.1997

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE
Designated Extension States:
AL LT LV MK RO SI

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(54) Protein-containing feedstuff and process for its manufacture

(57) A protein-containing feedstuff is produced by subjecting a material that contains vegetable protein to a heat-treatment; washing the resultant material one or more times, and preferably in a counter-current manner, with water, generally at pH of less than 7 and at a temperature of from 5 to 50°C; and subjecting the washed material to a farther heat-treatment, preferably a thermal drying step in which the protein-containing material may be dried to a moisture content of 12% by weight or less. Antinutritional factors such as mono-, di- and oligosaccharides may thereby be removed whilst retaining important nutrients such as isoflavones. Phytase may be added to the washwater in order to reduce the content of phytate in the product. Suitable starting materials include defatted soy flour, soy meal and texturized soy products. The feedstuff is suitable for the feeding not only of fish or crustaceans in aquaculture, but also of other livestock, for example piglets. The product may also be used for human nutrition.

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ground meal and of which 90% has a diameter of less than 45 μm , (c) ultra-finely grinding the first fines fraction so that 90% of the particles have a diameter of less than 20 μm , (d) air-classifying the ultra-finely ground material to produce a second coarse fraction and a second fines fraction, which latter is 50-90% by weight of the ground firstfines fraction and of which at least 80% of the particles are less than 20 μm in diameter, and (e) washing the second coarse fraction with water at a pH of 4 to 6, and separating a protein-containing residue from the liquid supernatant.

[0008] US-A-3,965,086 discloses a process for producing a protein concentrate which comprises (a) finely grinding an oilseed meal so that 90% of the particles thereby obtained are less than 100 μm in diameter and air classifying the ground meal to obtain a fraction characterised by its relatively high protein content and relatively low content of water-insoluble carbohydrate, and (b) washing that fraction in water at a pH of 4 to 6.

[0009] It is also known, however, that the digestibility of many vegetables, particularly the oilseed vegetables such as soybeans and other legumes, is interfered with by naturally occurring substances. The use of vegetable proteins in live-stock feeding is limited by those anti-nutritional factors.

[0010] One sector of the animal feed industry where vegetable protein sources are not commonly used is the aquaculture feed industry. The most important and mostly used protein source in aquaculture is fishmeal. Fishing and the processing of fish to fishmeal has been put under pressure, however, because along with the fish needed, smaller fish and other species are caught, which can endanger the survival of some fish populations.

[0011] On the other hand, an increase of fish consumption can be expected, mainly due to several health-related reasons. Fish has some nutritional advantages over meat, such as a higher amount of polyunsaturated fats or lipids and low cholesterol levels.

[0012] World production of fishmeal is quite constant and not expected to increase in the future. The amount of fishmeal needed to produce fish feeds could double in the next decade, if aquaculture continues to expand as predicted (Hardy 1995). (Bibliographic details of literature references herein are listed at the end of the description.) Therefore it is desirable to replace a part of or the entire, fishmeal fraction of a feed by a vegetable protein source. If a vegetable protein source is to replace fishmeal, it has to meet certain requirements. The quality and composition of the fishmeal should be taken as a starting point: thus, the most important requirements for application of this vegetable protein source in fishfeed would be the high protein content, the low amount of anti-nutritional factors, and the processability.

[0013] The most limiting factors in using for instance soybean meal as a fishmeal replacer, are the oligosaccharides, phytic acid and protease inhibitors. According to Arnesen *et al.* (1989) and Krogdahl (1989), oligosaccharides have a significant negative effect on the nutrient absorption, on the faecal dry matter excretion and on the digestibility of fat by salmonids. According to Spinelli *et al.* (1983), the role of phytic acid in reducing the bioavailability of minerals from soybean meal is well known and has been excellently reviewed. According to several authors (Sandholm *et al.*, 1976; Olli *et al.* 1989), salmonids are very sensitive to protease inhibitors. Protease inhibitors cause a reduction of the intestinal proteolytic activity, leading to an increase of the faecal excretion of protein.

[0014] Thus, when replacing fishmeal with a vegetable protein source, like soybean meal, it would be desirable to develop an efficient and effective process for treating vegetable protein sources in order to increase their digestibility and protein content. When replacing most fishmeal by such a vegetable protein source this could even be a prerequisite.

[0015] Many studies have been reported in which the replacement of fishmeal by different vegetable protein sources have been examined. Plant protein sources that can be used in fish feed are, for instance, rapeseed meal, soybean meal, soybean concentrate, soybean isolate, cottonseed meal, distillers' grain, and the like (Lim and Sessa (eds.), 1995). The quantities of the different types of protein that can be used in commercial fishfeed diets depend on several factors, including the nutritional value of the protein, the price of the protein, the presence of anti-nutritional factors in the protein source, and the ease with which the total feed may be pelletised (its "pelletisability"). The main reason for not using soybean isolate in fishfeed is the high price. Soybean concentrates could hitherto only be used to a limited extent, owing to the presence of anti-nutritional factors and to the poor pelletising properties.

SUMMARY OF THE INVENTION

[0016] In a first aspect, the present invention provides a process for the production of a protein-containing product, characterised in that a source of vegetable protein (a) is subjected to a heat treatment, (b) is afterwards washed in one or more steps with water at a temperature of 50°C or lower, and (c) is afterwards subjected to a further heat treatment.

[0017] In a second aspect, the present invention provides a protein-containing product that comprises at least 55-60% of vegetable protein on a dry weight basis, from 0 to 2% of simple sugars on a dry weight basis, a fat content of 0 to 1% on a dry weight basis, and a moisture content of 0 to 12% of the dry weight of the said product, the product exhibiting a trypsin inhibitor factor of 1.0 mg trypsin inhibited per gram of said product, or less. In general, the protein-containing product is also characterised by a content of phytate (expressed as phytic acid) of 0 to 0.5%, on a dry weight basis.

[0018] In a third aspect, the present invention provides a composition in the form of a food for human consumption or a feed for livestock, which composition comprises a protein-containing product that has been produced by a process

between 4.3 and 5.5. In certain other, and currently preferred, embodiments, the pH will be between 5.5 and 7. The water may be obtained from any supply that is suitable for use in the production of feedstuffs; for example, the water may be mains water, potable water from natural sources such as springs, desalinated seawater or distilled or deionised water. The pH of the washwater may be lowered to the desired pH value by the addition of an acid, preferably a food-grade or feed-grade acid. The acid may be an inorganic acid, for example sulphuric, nitric, phosphoric or hydrochloric acid, or an organic acid, for example citric or acetic acid. A mixture of two or more acids may, of course, be used.

[0027] Furthermore, phytase can, in general, be included in this water in at least one washing step, preferably in at least half of the washing steps and more preferably in all of the washing steps, so that during this process, the phytate-content of the soybean meal or other vegetable-protein source is lowered. Phytase of microbial origin is currently commercially available, e.g. from Messrs. Gist Brocades or Messrs. NOVO. The amount of phytase will generally be from 0 to 1% by weight relative to the weight of vegetable protein source, e.g. from 0.001 to 1.0%, typically 0.01 to 1.0%.

[0028] The pH which is recommended, in particular a pH below 7, is a compromise to achieve the following goals. A primary goal is that comparatively little protein, if any, will tend to dissolve in the washwater. A second goal is to keep microbial growth at a minimum. A third goal is to keep the phytase (if used) active, and a fourth goal is to make the washing process as effective as possible, especially with regard to meeting end-use requirements, for example when the product is to be used as a fishmeal replacer.

[0029] The washing of the defatted soybean meal or other vegetable-protein source may be carried out in any suitable manner, e.g. by continuous washing, batchwise washing, counter-current washing, co-current washing or centrifugal washing. It is preferably carried out counter-currently: a counter-current process is efficient and economical in that it permits removal of sugars with less water. In general, the washing will be effected in a plurality of steps, typically in three or more, e.g. up to seven, steps, preferably of 10 to 60, more preferably 15 to 30 minutes, e.g. about 20 minutes each. Washing may be effected in tanks. The ratio of water to soybean meal (or other vegetable protein source) is normally from 10:1 to 4:1, e.g. between 7 to 1 and 4 to 1, by weight. By choosing the right ratio of washwater:soybean meal (or other vegetable-protein source), the extraction can be optimized.

[0030] It has been found that when the temperature of the washwater does not exceed 20°C comparatively little protein, if any, will tend to dissolve into the washwater. If too much protein were to dissolve, this would lower the protein content of the end-product and lower the yield. Nonetheless, in view of the improved processability (discussed in greater detail hereinafter) that may be obtained in accordance with the present invention, it may on occasion be tolerable to employ a higher temperature in the washing stage, especially a temperature not exceeding 35°C. Provided that the water can still be used to effect washing, there appears to be no precise lower temperature limit, although phytase activity will be lower at low temperature. It may prove convenient to use washwater having a temperature higher than 5°C.

[0031] In certain preferred embodiments, the toasted defatted soybean meal (or other vegetable-protein source) is washed in a plurality of steps and the washwater used in each washing step has a lower content (by weight, on a dry matter basis) of soluble substances - in particular, soluble substances extracted from the toasted defatted soybean meal (or other vegetable-protein source) - than the washwater used in the preceding step. In other words, the defatted soybean meal (or other vegetable-protein source) is washed in a series of steps using washwater of progressively lower contents of soluble substances, in particular extracted soluble substances. Thus, for example, during the last step, the toasted defatted soybean meal (or other vegetable-protein source) will normally be washed with fresh washwater. The water which is left or recovered after this step is used for the washstep before the last one in another cycle, i.e. in the treatment of another batch of vegetable-protein source, and so on, so that the first washstep is carried out with the least-fresh water.

[0032] After every washstep, the washed material and the washwater are generally separated. In this way, most water can be used again. Preferably, the toasted defatted soybean meal (or other vegetable-protein source) is partially dehydrated by means of hydro-cyclones (a battery of at least three cyclones will normally be used), although other means, such as a centrifuging and pressing step, may be used instead. The dry-matter content of the washed product after separation of the washwater is generally from 5 to 25%, preferably from 10 to 20%, by weight. By washing with water with a pH between 4 and 7 and phytase, all oligosaccharides and most phytate will in general be mobilized into the water. The smaller hydrophilic proteins also dissolve into the water. Beneficial factors such as isoflavones will stay in the soybean meal (or other vegetable-protein source). Because of the fact that most small hydrophilic proteins are already dissolved, the leak-out of proteins of the final product, especially when used in aquaculture as a fishfeed, will be minimal: because there is less loss of protein from the feed, the use of such a fishfeed will be more efficient.

[0033] The vegetable-protein source, after it has been subjected to the preliminary heat treatment and washing, is subjected to a further heat treatment, which conveniently may comprise, or be constituted by, a thermal drying operation. In general, the washed material is subjected, in this further heat treatment, to a temperature of at least 80°C, preferably at least 90°C; in general, the temperature will not exceed 150°C, and is preferably no higher than 120°C. Typically, the vegetable-protein source will attain a temperature of 100-105°C. The heat-treatment is normally carried out such that it results in a product in which the protein is farther denatured, preferably strongly denatured: in general,

these isoflavones will be extracted. By washing in accordance with the present process however, these isoflavones will be preserved: isoflavones are not influenced by this process, so that the content of isoflavones in this product can be kept at the highest level. The isoflavones are anti-oxidizing agents and have several health-related advantages.

[0042] By adding microbial phytase to the washwater, the total amount of phytate can be reduced, so that the availability of phosphate and minerals will increase.

[0043] It has been found that the treatment of vegetable protein sources in accordance with this invention increases the protein content and digestibility thereof to an extent that permits their use as or in feedstuffs. The expression "feedstuffs" is to be construed broadly to include a feed, or a constituent nutrient of a feed. The feedstuff may be applied to the nutrition of livestock (including monogastric animals, including mammals, such as pigs and their young, and also including poultry and the like; other farm animals; fish, for example salmonids such as salmon or trout; and crustaceans, for example shrimps), pets, working animals, and animals kept for sporting purposes. The concentrated protein product can also be used as or in a food for humans.

[0044] In fishfeed, this concentrated protein product can replace at least 20 percent fishmeal. Another advantage of the use of this concentrated protein product is that it has good pelletising properties: thus, when making a fishfeed containing 20% of the concentrated protein product of this invention pelletising is very satisfactory. Furthermore, it has been found that the final feed does not fall apart too quickly when put into water, and that it can be used in pneumatic feeding systems. Because of the texture and the fact that some hydrophilic proteins are washed out, the oil holding capacity of this feed ingredient is very good, compared with other protein sources. This makes it very easy to add fish oil, oil from other origins, fractions of oils or derivatives of oils to this protein source at high concentrations, so that the manufacturing of the final feed is relatively simple. The protein source can be adapted to specific applications, e.g. in fishfeed, by adding for instance specific and/or modified fatty acids, bio-colorants, proteins, amino acids, carbohydrates or lipids.

[0045] This proteinaceous product can not only be used in aquaculture, but also in many other applications which may include the use in pet food, pig growth starter and in food for human consumption, also because the texture of this product is very suitable. The amounts of this concentrated vegetable protein source are not restricted to a certain level, because virtually all of the antinutritional sugars are washed out.

[0046] Foods for human consumption may include the following: meat imitations, protein-rich beverages, sausages, imitation cheese, cow-milk replacers (for people that are allergic to cow-milk) and the like. The amount of this protein concentrate which could be incorporated into these products depends on the food-product formulation and properties.

EXAMPLES

[0047] The following examples are not intended to limit or narrow the invention herein, but are for illustrative purposes only. As elsewhere in this specification, percentages and parts are by weight unless otherwise stated.

Example 1:

[0048] The following table illustrates the physical and chemical properties of the concentrated soy protein product (A1 and A2), compared with those of soybean concentrate (SBC), defatted soybean meal (SBM) and texturized soybean meal (TSP). Note that the concentrated soy protein products are prepared under different conditions, from different raw materials. A1 is produced from texturized soybean meal, A2 is produced from toasted defatted soybean meal. During the production of A2, also phytase was added.

TABLE 1

Physical and Chemical Properties of Soy Products					
% of dry solids	SBC	SBM	TSP	A1	A2
Protein	68	54	49	73	69
Fat	0.3	1.5	0.5	0.3	0.6
Galactose	<0.3	<0.5	<0.5	<0.3	<0.3
Glucose	<0.3	<0.5	<0.5	<0.3	<0.3
Fructose	<0.3	<0.5	<0.5	<0.3	<0.3
Saccharose	<0.3	5.0	5.56	<0.3	<0.3
Raffinose	<0.3	1.0	1.04	<0.3	<0.3

[0050] From this test can be concluded that the diets which contain the experimental products (A1 and A2) give the same performance as the other diets. The protein- and fat digestibility of both A1 and A2-based diets tends to be somewhat better than the other diets, the feed consumption tends to be about the same, and the feed conversion and specific growth rate somewhat less. No significant differences were found.

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Example 3:

[0051] Two four-week studies with piglets were conducted. In the first study, texturized soybean meal is compared with two other protein sources that are used in piglet feed. These are skimmed milkpowder and soybean meal. In the second study, the texturized soybean meal is compared with experimental product A1. The feed is produced as a pellet (4 mm). The pellets are produced with the addition of steam. Pelleting temperature is about 70°C. In both studies Hypor piglets, weaned at about 26 days, are used. Both experiments were designed as split litter trials (3 to 5 piglets per half litter). Per comparison, at least 15 complete litters were used.

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[0052] In Table 3A, the composition and analysis of the experimental diets are given.

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TABLE 3B: Technical results of the first study in which texturized soybean meal-diet is compared to the skimmed milkpowder-diet and separately compared to the soybean meal-diet

	TRIAL 1		TRIAL 2			
	TSP versus SMP		TSP versus SBM		TSP versus AI	
	TSP-diet	SMP-diet	TSP-diet	SBM-diet	TSP-diet	AI-diet
n(number of litter halves)	17	17	15	15	15	15
Weight at onset trial (kg)	7.9	7.8	8.1	8.0	8.2	8.2
Weight after 14 days	10.2	10.4	9.9	9.9	10.6	11.0
Weight after 28 days	16.8	16.8	16.5	16.8	17.9	18.7
Period 1 (0-14 days)						
Growth (g/day)	167	180	136	131	174	201
Feed intake (g/day)	296	308	264	267	285	302
Feed efficiency	0.552	0.579	0.495	0.487	0.606	0.656
Feed conversion ratio	1.8	1.7	2.0	2.1	1.7	1.5
Diarrhoea occurrence (%) [*]	20.6	10.9	20.5	28.1	16.7	17.1
Diarrhoea severity ²	1.7	1.5	1.6	1.7	1.4	1.7
Period 2 (15-28 days)						
Growth (g/day)	469	460	489	474	517	548
Feed intake (g/day)	751	745	751	747	796	821
Feed efficiency	0.625	0.620	0.652	0.635	0.647	0.667
Feed conversion ratio	1.6	1.6	1.5	1.6	1.6	1.5
Diarrhoea occurrence (%) [*]	6.3	4.2	5.2	6.2	10.5	7.1
Diarrhoea severity ²	1.5	1.5	1.6	1.4	1.3	1.5
Total period (0-28 days)						
Growth (g/day)	318	320	312	303	346	375
Feed intake (g/day)	523	527	508	507	541	562
Feed efficiency	0.604	0.605	0.613	0.596	0.637	0.666
Feed conversion ratio	1.7	1.7	1.6	1.7	1.6	1.5
Diarrhoea occurrence (%) [*]	13.5	7.6	12.9	17.1	13.6	12.1
Diarrhoea severity ²	1.6	1.5	1.6	1.6	1.4	1.7

^{*}: Diarrhoea occurrence (%):

$$\left(\frac{\text{times diarrhoea scored}}{(\text{number of pens} \times \text{number of days})} \right) \times 100^*$$

²: Diarrhoea severity:

$$\left(\frac{(\text{flat manure} \times 1 + \text{diarrhoea} \times 2 + \text{watery faeces} \times 3)}{(\text{times diarrhoea scored})} \right)$$

[0054] Texturized soybean meal leads to equal technical results, but to significantly more diarrhoea during the first two weeks after weaning, compared with skimmed milkpowder. Compared with standard soybean meal, the use of tex-

protein concentrate having a very low content of phytate.

Example 7:

5 [0066] 150 kg of soy flakes were put into a tank and 750 litres of fresh tap water were added. This was mixed for 30 minutes with the help of a top mixer (speed 340 rpm). This was necessary because the product settles easily. After washing, the slurry was filtered in a finisher (FMC model 35, screen size 0.51 mm), with a pressure on the outlet of 40 bar. The washed soy flakes were recycled for farther washing and the washwater was sent to a 5 effects falling-film evaporator. To the recycled, washed soy flakes, water was added up to the final volume of about 850 litres and this was
10 mixed again for 30 minutes, as described above. After this wash step, another wash step was carried out.

[0067] After the last dewatering step, the soy flakes were dried in a direct fire drier, with a good control on the temperature. Drying (exit air) temperature was between 93.3 to 143.3°C. The product was dried up to a moisture content of at maximum 10%.

15 [0068] The same experiment was carried out with acidic washwater. Sulphuric acid was added to the water, so that the pH of the mixture soy flakes and water was 5.0. At every new wash step, the pH was adjusted to 5.0. In the table below, the chemical analyses of these test products are given (percentages on as is basis).

Drying temperature (°C)	pH	Moisture (%)	Protein (%)	Protein % DW-basis	Fibre (%)	PDI	Ash (%)	TIA (mg/g)
Starting material	6.8	11.3	46.5	52.4	4.5	23	5.7	1.5
93.3	7.2	13.7	52.5	60.8	8.0	8	3.5	0.3
110	7.2	9.1	55.9	61.4	8.7	7	3.7	0.4
121.1	7.1	5.2	58.3	61.5	8.4	6	3.9	0.3
143.3	7.1	4.1	55.7	58.1	9.8	5	4.1	0.3
Starting material	6.8	11.2	47.3	53.3	4.1	24	5.9	0.7
110	4.5	4.2	64.3	67.1	6.8	1	5.7	0.3
110	4.8	5.1	61.5	64.8	8.3	1	3.9	0.3

Example 8:

40 [0069] The technical and physical properties of product A2 were tested. Pellet hardness, pellet length, expansion, and processability were measured and the final feeds were also visually checked. As references, traditional soy concentrate and fishmeal were taken into account. The inclusion rates of product A2 in the final feeds were 10, 15, 20 and 25% of the total feed. The aim of this test was to conclude whether A2 had the same technical effect as fishmeal (as this is not the case with soy concentrates).

45 [0070] As earlier tests have shown, traditional soy concentrate is a difficult raw material in fishfeed production. This was not the case with the A2 product however. The binding properties of the A2 product are as good as the binding properties of fishmeal A2 also gave the same expansion as fishmeal. The latter is important in order to get enough fat into the pellets (oil-holding capacity). Both these parameters limit the usage of traditional soy concentrates in fishfeed production. The conclusion of these tests was that the A2 product has much better technical effects than traditional soya concentrates and is as good as fishmeal.

50 [0071] Thus, although the ease with which feeds containing the present vegetable-protein concentrates can be pelleted may depend also upon the other ingredients, tests have shown that the incorporation of concentrated vegetable-protein products of this invention into typical feeds at a level of more than 5%, e.g. more than 10%, such as 15% or more, or even 20% to 25% by weight of the final feed does not present a problem with regard to pelletisation. This represents a distinct improvement over conventional soya products, which cannot successfully be incorporated at levels
55 higher than 5% by weight.

[0072] It will of course be understood that the present invention has been described above purely by way of example and that modifications of detail can be made within the scope of the invention.

11. A process according to any of claims 1 to 10, in which from 4 to 10 parts by weight of water are used in the or each washing step per part of the source of vegetable protein.
12. A process according to any of claims 1 to 11 in which the washing is carried out at a temperature of up to 35°C, e.g. at a temperature of up to 20°C.
13. A process according to any of claims 1 to 12, in which, in the said first heat treatment, the vegetable-protein source is heated sufficiently to reduce its protein dispersibility index to a value that is less than 35%, e.g. less than 30%.
14. A process according to any of claims 1 to 13, in which, in said first heat treatment, the vegetable protein source is heated to a temperature of from 80°C to 150°C.
15. A process according to any of claims 1 to 14, in which the said first heat treatment is carried out in a desolventizing-toasting apparatus.
16. A process according to any of claims 1 to 15, in which, in the said farther heat treatment, the vegetable-protein source is heated sufficiently to reduce its protein dispersibility index to a value that is less than 20%, e.g. less than 15%.
17. A process according to any of claims 1 to 16, in which, in the said further heat treatment, the vegetable-protein source is heated to a temperature of from 80° to 150°C.
18. A process according to any of claims 1 to 17, in which the washed material is at least partially dried.
19. A process according to claim 18, in which the drying of the washed material includes the step of decanting off water remaining from the last washing step.
20. A process according to claim 18 or 19, in which the washed material is subjected to a thermal drying step.
21. A process according to any of claims 1 to 20, in which the said farther heat treatment comprises a thermal drying step.
22. A process according to claim 20 or 21 in which the thermal drying step is effected in a fluidized bed dryer or a rotating drum dryer.
23. A process according to any of claims 18 to 22, in which the washed material is dried to a moisture content of 12% by weight or less.
24. A process according to any of claims 1 to 23, in which the source of vegetable protein which is subjected to the washing steps is obtained from soybeans and/or other legumes and/or other oilseed vegetables.
25. A process according to claim 24, in which the said source of vegetable protein is defatted soy meal, defatted soy flour, defatted soybean meal, a texturized soy product, or a mixture of any two or more of these.
26. A protein-containing product which comprises at least 60% of vegetable protein on a dry weight basis, from 0 to 2% of simple sugars, on a dry weight basis, a fat content of 0 to 1% on a dry weight basis, and a moisture content of 0 to 12% of the total weight of the product, and exhibits a trypsin inhibitor factor of 1.0 mg, or less, trypsin inhibited per gram of product.
27. A protein-containing product according to claim 26 having a content of phytate (calculated as phytic acid) of 0 to 0.5% by weight.
28. A protein-containing product according to claim 26 or 27, in which the content of isoflavones is 900 ppm or greater.
29. A composition in the form of a food for human consumption or a feed for livestock, which composition comprises a protein-containing product that has been produced by a process according to any one of claims 1 to 25 or a protein-containing product according to claim 26, 27 or 28, in admixture with at least one other ingredient selected from sources of nutrition and edible additives.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 97 31 0497

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 10 June 1998	Examiner Dekeirel, M
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